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(Quarterly Status Report)

LIFE IN EXTRATERRESTRIAL ENVIRONMENTS

Contract No. NASr-22

National Aeronautics
and Space Administration
Washington, D.C.

IIT RESEARCH INSTITUTE

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August 15 through November 15, 1968

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I. INTRODUCTION

Research work the past quarter involved experiments on the survival of microorganisms airborne in dust clouds. The viable cell count of Bacillus cereus and B. subtilis decreased 99% and Staphylococcus aureus decreased 99.9% while suspended in simulated Martian dust clouds for 28 days. The humidity and temperature of the test environment ranged from 40 to 60% RH and from 20 to 25°C, respectively. Serratia marcescens did not survive one day in the test environment.

The time factor and initial cell populations must be considered with decreasing viability determinations. In the present studies the decreased viability took place within 14 days without any further significant change. If bacterial populations of 10^4 to 10^6 cells are considered, then the numbers of survivors are important to the contamination of Mars since populations between 10 and 100 cells of some organisms can survive and grow in a simulated Martian environment.

In addition, superimposing daily freeze-thaw cycles, different relative humidities, gaseous composition, and barometric pressure may markedly alter survival of an organism based upon our previous studies.

A large portion of the past quarter was spent assembling the rotating chamber inside an environmental chamber to conduct the daily freeze-thaw cycles. This was necessary since the electric motor could not operate at the -65°C temperature required to simulate the Martian night. At the present time the rotating chamber is geared to a flexible coupling and shaft which in turn is connected to the electric motor outside of the environmental chamber. Another problem encountered was with the bearings that supported the rotating chamber. The bearings would not function at the low temperature. A modified chamber design has been completed that uses low temperature grease-packed bearings that should solve the low temperature compatibility problem.

Another portion of work was involved with calibrating the intensity of the ultraviolet lamp at different wavelengths. The lamp (Hanovia type A lamp No. 673A, 550 watts) emits approximately 2.5 times the UV intensity over the 2400 to 2800 A wavelength band calculated for the surface of Mars. The increased intensity of the lamp compared to the UV flux at the surface of Mars will be compensated for by either shortening the time of lamp operation or increasing the distance of the lamp from the rotating chamber.

Salt tolerant, facultative anaerobic bacilli and cocci were isolated from different soils. There appears to be a greater correlation between the type soil the organism was isolated from, rather than the minimum a_w requirement, and the ability to grow in a simulated Martian environment. Larger numbers of bacillus and coccus isolates from desert and tundra soils were able to grow in a simulated Martian environment than isolates from temperate climate soils.

II. EXPERIMENTAL METHODS

The rotating chamber used in the experiments was described in detail in Reports No. IITRI-L6023-13 and -14. *S. aureus*, *S. marcescens*, and spores of *B. cereus* and *B. subtilis* were used in the studies to determine the abrasive effect of dust clouds on the survival of these organisms.

Methods used for preparation of stock cultures were described in Report No. IITRI-L6023-5. Stock cultures of spores were stored at 4°C until used while stock cultures of *S. aureus* and *S. marcescens* were prepared fresh for each experiment.

The method for isolating salt tolerant bacteria from soils was described in Report No. IITRI-L6023-14. The soils were obtained from the following locations:

1. Desert Soils
    a. Libyan Desert, Libya
    b. Mecca Hills, California
    c. Sonoran Desert, Arizona
    d. White Sands National Monument, New Mexico
2. Tundra Soils
    a. Rocky Mountain National Park, Colorado
    b. White Mountains, California
3. Prairie- and tree-type soils from Kane County, Illinois.

The isolates were grouped according to their colonial and cellular morphologies and minimum a_w requirement. Biochemical tests are nearing completion.

III. RESULTS AND DISCUSSION

A. Rotating Chamber Studies

Figure 1 shows the survival of *B. cereus*, *B. subtilis*, and *S. aureus* in simulated Martian dust clouds over a 28-day period. The humidity and temperature were not controlled and varied with room conditions. The relative humidity ranged from 40 to 60% RH and the temperature from 20 to 25°C.

Cells of *S. aureus* and *S. marcescens* and spores of *B. cereus* and *B. subtilis* were allowed to equilibrate overnight in a 5% (w/v) solution of i-inositol before inoculation into the soil.

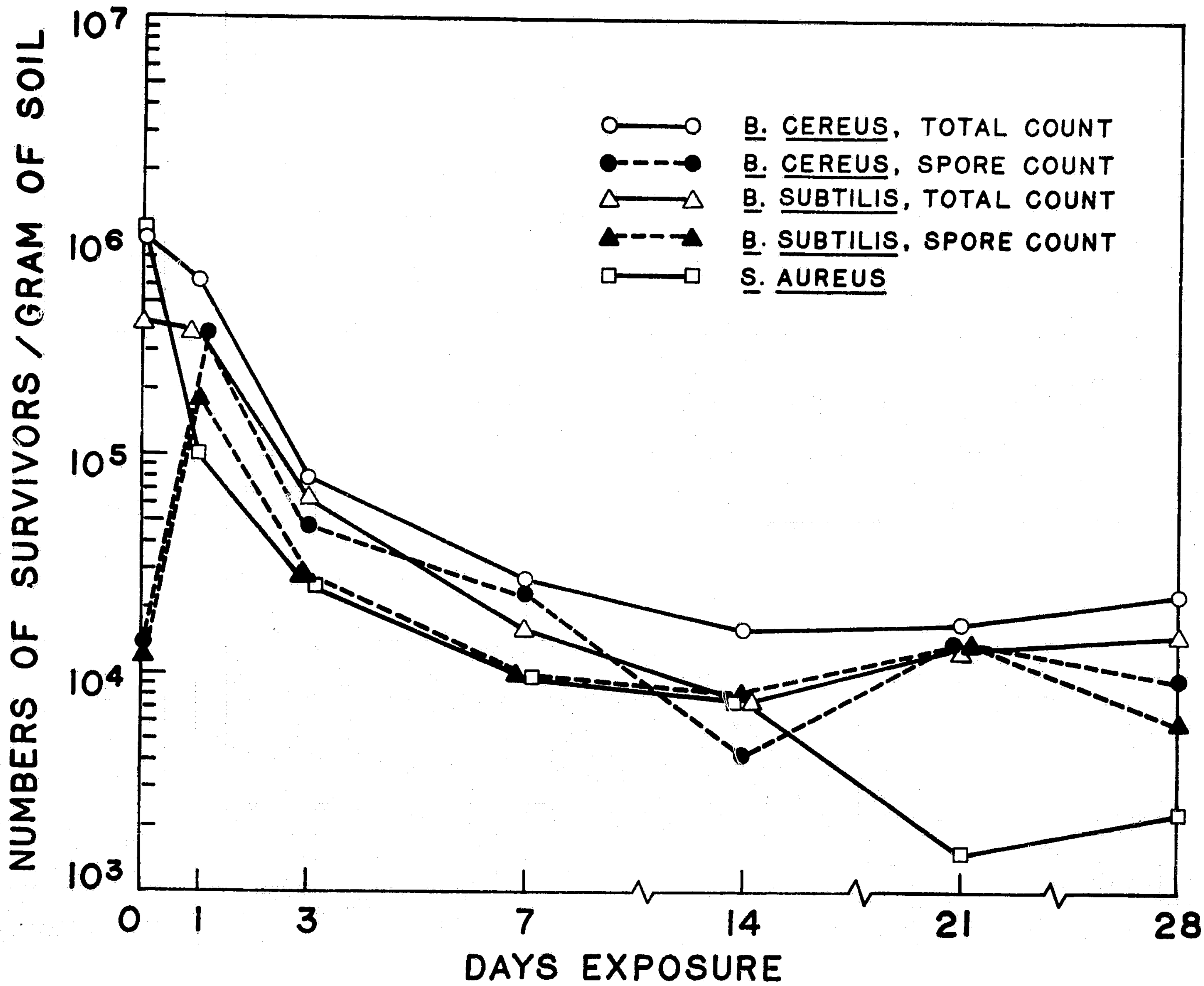

FIG. I THE SURVIVAL OF SELECTED BACTERIA IN SIMULATED MARTIAN DUST CLOUDS.

Inositol was used to prevent loss of bound water and subsequent cell death (S. J. Webb, Bound Water in Biological Integrity, Charles C. Thomas, Pub. Springfield, Ill., 1965). There was no apparent effect of inositol since the survival curves with and without inositol were similar (IITRI Report No. L6023-14).

The survival of B. cereus and B. subtilis was similar. Cell viabilities decreased to 1% by 14 days in the test environment without any further remarkable change. The survival of S. aureus decreased at about the same rate as B. cereus and B. subtilis up to 14 days followed by a further decrease during the remaining days of the 28 day exposure.

S. marcescens was extremely sensitive to the test environment. Viable cells were not recovered after 1 day in the test environment.

The decreased viability that occurred within 14 days without any additional decrease still imposes spacecraft sterilization constraints to guard against the contamination of Mars since significant numbers of bacteria would survive from initial populations of 10^4 to 10^6 viable cells. Previous studies (Report No. IITRI-L6023-12) found that initial cell populations between 10 and 100 cells would survive and grow in a simulated Martian environment.

B. Salt Tolerant Isolates

The salt tolerant isolates were grouped according to their cellular morphology and minimum a_w requirement (Table 1). The term salt tolerant is given to those organisms that grew on trypticase soy agar (BBL) with 7.5% NaCl (w/v) added.

Table 1

NUMBER OF SALT TOLERANT ORGANISMS ISOLATED FROM SOILS

Cell Morphology	Minimum a_w Requirement			Total Number of Isolates
	0.90	0.86	0.84	
Bacillus	26	19	9	54
Coccus	4	15	5	24

There are five less bacillus and fifteen less coccus isolates then given in the last report (Report No. IITRI-L6023-14). This was the result of discarding duplicate cultures and a few cultures not remaining viable. The isolates that grew at low a_ws were not characteristic of any particular soil type. Organisms with low a_w requirement were isolated from temperate climate soils such as the prairie-and tree-type soils of Illinois as well as the desert and tundra soils.

All 78 isolates were tested for their ability to survive and grow in a simulated Martian environment. The simulated Martian environment was as follows: 67% carbon dioxide, 20% nitrogen, 3% argon at 15 mb pressure; daily freeze-thaw cycle (16-hr at -65°C and 8-hr at 30°C); and an a_w of 0.99. Viable cell counts were determined before and after 7 days in the test environment. The cultures that had a final viable cell count 1-log or greater than the initial count were scored as growing in the simulated Martian environment. Table 2 presents this data.

Table 2

THE SURVIVAL AND GROWTH OF SALT TOLERANT ORGANISMS IN A SIMULATED MARTIAN ENVIRONMENT

Cell Morphology	Minimum a_w Requirement 0.90 Survived	0.90 Grew	0.86 Survived	0.86 Grew	0.84 Survived	0.84 Grew
Bacillus	26	7	19	0	9	3
Coccus	4	3	15	11	5	3

The isolates that grew in the simulated Martian environment were isolated, in general, from soils from severe terrestrial environments: White Mountains, Mecca Hills, Catalina Mountains, White Sands, and Libyan Desert soils. A greater proportion of the coccus isolates were able to grow in the simulated Martian environment than the bacillus isolates.

There apparently is no relationship between low a_w requirement and the ability to grow in the simulated Martian environment: with the exception of the bacillus isolates with minimum a_w of 0.86 the ratio of the isolates that grew compared to the total number that survived is similar within a cell morphological group irrespective of the minimum a_w requirement.

This collection of organisms from different soils establishes the widespread distribution of diverse morphological types that possess salt tolerance and are able to survive and grow in simulated Martian environments.

IV. SUMMARY

The viable cell count of B. cereus and B. subtilis decreased 99% and S. aureus decreased 99.9% during a 28-day exposure to simulated Martian dust clouds at ambient humidity and temperature conditions. The numbers surviving could still be significant depending upon the particular organism and the numbers initially present. S. marcescens did not survive one day exposure to the test environment. Definite conclusions on the survival of microorganisms in simulated Martian dust clouds cannot be made until other environmental conditions such as daily freeze-thaw cycle, moisture concentration, gaseous composition, and barometric pressure are investigated.

An additional environmental condition to consider would be an interrupted cycle. This type of an approach is directed toward the possibility of organisms being deposited at a location on Mars where growth would not occur because of insufficient moisture, organic material, or length of the daily freeze; but upon being redeposited at another site, as a result of wind activity, growth could occur. This condition could be studied in the following manner: when the number of organisms decreased 90 to 99% in the airborne soil particles, conditions permitting the growth of the organisms would be initiated by increasing the moisture or organic material concentration or by shortening the length of the daily freeze. After growth has occurred the original set of environmental conditions would be reestablished.

The properties of 78 salt tolerant bacteria isolated from a variety of soils are being investigated. There does not appear to be any relationship between the minimum a_w of an organism and the type of soil from which it was isolated. However, there does appear to be a relationship between the ability of an organism to grow in a simulated Martian environment and the type soil from which it was isolated. The greatest number of organisms able to grow in a simulated Martian environment were isolated from soils taken from severe terrestrial environments.

V. PERSONNEL AND RECORDS

The experiments were planned with the counsel of Dr. E. J. Hawrylewicz and the technical assistance of Mr. B. T. Anderson and Mrs. M. L. Cephus.

Experimental data are recorded in IITRI Logboosk C18591, C18833, and C19044.

Respectfully submitted,

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